IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Miri Seiberg, et al. Serial No. 09/206,249 Filed: December 7, 1998 Art Unit: 1654 Examiner: M. Meller Attorney Docket No.: JBP 438	METHODS FOR REGULATING PHAGOCYTOSIS AND ICAM-1 EXPRESSION

DECLARATION OF MIRI SEIBERG, PH.D.

I, Miri Seiberg, am a Distinguished Research Fellow in the Skin Research Center at Johnson & Johnson Consumer Companies, Inc. My education includes a Ph.D. in Molecular Biology from The Weizmann Institute of Science, Rehovot, Israel, in collaboration with Princeton University, Princeton, NJ and a B. S. in Life Sciences from Tel-Aviv University, Tel-Aviv, Israel. My curriculum vitae is attached hereto as Exhibit 1.

1. All protein molecules are composed of polypeptide chains of α-amino acids. Proteins are defined by both (1) their chemical structure, which includes its substituent amino acids as well as their unique conformation and (2) their biological function. A protein's biological function or activity requires the presence of both its chemical structure and conformation. (Biochemistry, A. L. Lehninger, 1975, p. 62- 66).

2. Proteins are said to be "denatured" when their physical and physiological properties are changed such that they lose their activity. Such change is generally due to a change in a protein's chemical structure and/or conformation. Protein denaturation and the consequent loss of biological activity are not related to the source of the protein or to their origin, and are described in biochemistry textbooks (e.g. Biochemistry, A. L. Lehninger, 1975, p.62-63).

3. Those knowledgeable about protein activity at the time the invention was made were aware that proteins are denatured in the presence of organic solvents. The effect of organic chemicals on protein denaturation has been studied for decades. A 1975 publication from Malveev describes the dependence of denaturation time on organic solvent concentrations. Extraction with organic solvents was shown to denature many proteins (Sikorski and Naczk, 1981). In 1984, Benedek et al measured the kinetics of denaturation of several proteins, including soybean trypsin inhibitor (STI), as a function of the organic modifier employed. Khmelnitsky et al (1991) documented the denaturation of several proteins by a broad series of organic solvents of different nature. van Erp et al (1991) developed a theoretical model, based

on a generally accepted notion that the destruction of the protein hydration shell is one of the main reasons for protein denaturation by organic solvents. These studies document that proteins (including STI) are denatured in the presence of organic solvents. Copies of the foregoing references are attached hereto as Exhibit 2.

4. Genistein is an isoflavone. Those knowledgeable of the process of isolation and purification of isoflavones are aware that the extraction of genistein from soybeans requires organic solvents. This solvent extraction knowledge was used, e.g. in United States Patent 5,679,806, in purifying isoflavones by using three steps of solvent extraction.

5. During such organic extraction processes, STI (and all other proteins) separates into the aqueous phase, which is removed from the final isoflavone preparation. As indicated above, residual STI that might have contaminated the organic phase of the genistein preparation would be denatured, and therefore inactive due to the presence of organic solvents. Therefore, a genistein preparation extracted from soy cannot contain even residual amounts of non-denatured and active STI.

6. A diffusion coefficient is a constant of proportionality that represents the amount of a substance diffusing across a unit area, through a unit concentration gradient, in unit time. Those knowledgeable about protein biophysical properties are aware that even decades ago it was possible to calculate and to measure the diffusion coefficients of proteins. It is known that the diffusion coefficients for biological molecules normally range from 10^{-11} to 10^{-10} m^2/s. (Fick's law of diffusion).

7. In 1948, Kunitz (attached hereto as Exhibit 3) measured the biophysical properties of STI. He found that the diffusion coefficient of STI is extremely low, therefore it was measured using a modified time unit, replacing the standard "per second" unit with a "per day" unit. Kunitz found that the diffusion coefficient of STI is 0.07-0.08 cm^2/day, at 24 degrees centigrade. It is obvious to those of ordinary skill in the art from this diffusion coefficient that STI will not diffuse out of the soybean into any soaking liquid.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Miri Seiberg

3/30/08

Date